

# Interleukin-8 Expressed in the Granulocytes of the Eye in a Patient with Behçet's Disease Complicated by Lens-induced Endophthalmitis

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**Background:** Interleukin-8 (IL-8) is believed to be involved in the progression of intraocular inflammation. We sought the source of IL-8 in the enucleated eye of the present patient.

**Case:** A 40-year-old Japanese man was diagnosed as having Behçet's disease. His vision deteriorated due to persistent uveitis and secondary glaucoma. His left eye had lens-induced endophthalmitis.

**Observations:** The left eye had to be enucleated, and it was investigated by an immunohistochemical analysis using antibodies for CD 1a (dendritic cells), CD 3 (T cells), CD 68 (monocytes/macrophages), interferon- $\gamma$ , or IL-8. Fibrovascular tissue had formed on and beneath the lens where inflammatory cells had infiltrated. Most of the mononuclear inflammatory cells were T cells. A large number of macrophages were observed especially around the lens. Interferon- $\gamma$ -positive cells were scattered, while IL-8 was observed only in the accumulated granulocytes, but not in either mononuclear cells or macrophages.

**Conclusion:** IL-8 is thus considered to play a role in the progression of intraocular inflammation, and granulocytes are thought to be a possible source of IL-8 in endophthalmitis. **Jpn J Ophthalmol 2003;47:548–550** © 2003 Japanese Ophthalmological Society

**Key Words:** Behçet's disease, endophthalmitis, granulocytes, interleukin-8.

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## Introduction

Interleukin-8 (IL-8) is a prototypical chemokine (C-X-C-subfamily) and a potent stimulator of polymorphonuclear leukocyte chemotaxis and activation, as well as other proinflammatory effects.<sup>1</sup> Regarding intraocular inflammation, the amount of IL-8 increased in murine lipopolysaccharide-induced uveitis while endotoxin-induced uveitis was inhibited by a neutralizing antibody for IL-8 in rabbits.<sup>2,3</sup> Several clinical studies have shown that the serum IL-8 level increases in uveitis, which is considered to be related to the activity of intraocular inflammation.<sup>4</sup> We carried out an immunohistochemical

study of the enucleated eye with uveitis and tried to identify the cellular source of IL-8 in intraocular inflammation.

## Case Report

A 40-year-old Japanese male patient with HLA-B51 had a 4-year history of Behçet's disease with recurrent oral ulcers, genital ulcers, and synovitis. In February 1993, when he was 36 years old, he began to complain of a visual disturbance in his left eye, which was diagnosed to be ocular Behçet's disease. His corrected visual acuities were 20/20 OD and 20/300 OS. An ocular examination showed various ocular complications such as iritis, hypopyon, vitreous cellular infiltration, retinal exudates, and optic atrophy in his left eye. He was given only topical steroid therapy because he did not have

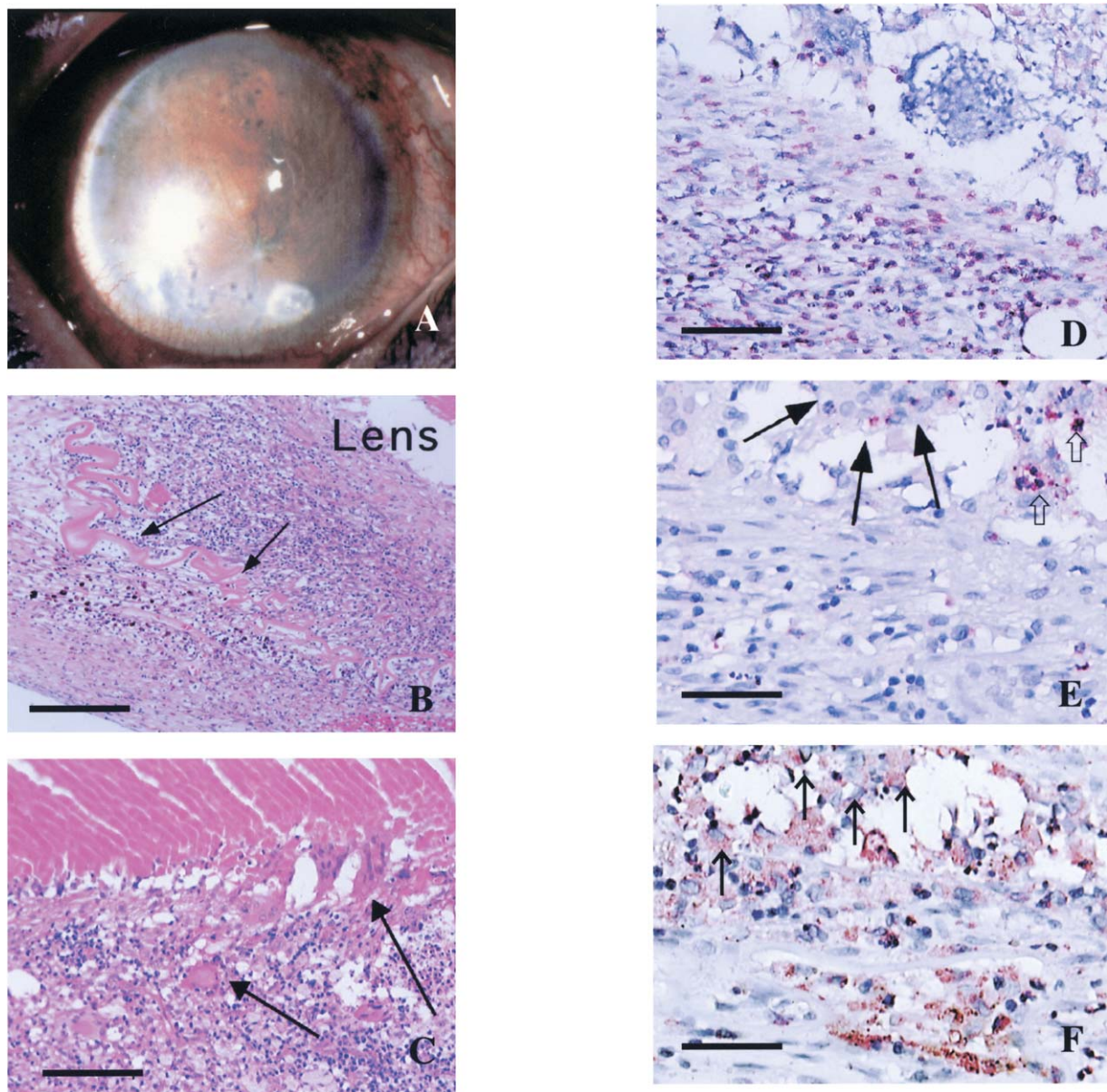
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ophthalmic examinations and therapy periodically. Vision in his left eye gradually deteriorated and the ocular fundus could not be observed because the pupil had closed permanently (Figure 1A). In September 1997, he had

severe uveitis and secondary glaucoma. Two months later, November 11, 1997, we obtained informed consent from the patient and his left eye was enucleated due to the persistent ocular pain.



**Figure 1.** (A) Photograph of affected eye of patient with Behçet's disease and lens-induced endophthalmitis. The pupil has closed due to the posterior synechiae of the iris. (B), (C) Light microscopic photographs in enucleated eye. A thick fibrous membrane formed on both the anterior and posterior surfaces of the lens and a disrupted lens capsule was seen (B, arrows) while multinuclear giant cells (C, arrows) were found adjacent to the lens material (hematoxylin and eosin stain. Bars = B: 500  $\mu$ m, C: 200  $\mu$ m.) (D–F) Results of immunohistochemical study of enucleated eye. Most of the infiltrated cells are positive for anti-CD3 antibody (D). IL-8-positive cells are exclusively found adjacent to the lens (E). CD68-positive macrophages are diffusely present around the lens (F, arrows). Sequential sections (E: IL-8, F: CD68) showed that IL-8 is stained in the granulocytes (E, white arrows, in upper right of figure), but no indication of IL-8 cells is seen in multinuclear giant cells (E, arrows). Positive staining is demonstrated by a reddish color. (Avidin-biotin peroxidase complex method. Bars = D: 100  $\mu$ m and E, F: 50  $\mu$ m).

## Immunohistochemical Study

Immunohistochemical study was carried out on paraffin sections of the enucleated eye, using the previously described method.<sup>5</sup> The primary monoclonal antibodies used in this study were anti-IL-8 (Genzyme, Cambridge, MA, USA), anti-human interferon- $\gamma$  (Genzyme), anti-human thymocyte CD1a (Dako, Glostrup, Denmark), anti-human T cell CD3 (Dako), anti-human macrophage CD68 (Dako), and isotype-matched control (PharMingen, San Diego, CA, USA).

## Results

The retina was totally detached, and the iris and the anterior choroid had been infiltrated by mononuclear cells. A thick fibrous membrane had formed on both the anterior and the posterior surface of the lens (Figure 1B). The lens capsule had been destroyed by inflammatory cell infiltration associated with multinuclear giant cells (Figure 1C). Many of the mononuclear inflammatory cells were positive for anti-T-cell antibody CD3 (70%, determined by counting the number of CD3-positive cells/total inflammatory cells per microscopic field (Figure 1D)). IL-8 was stained in the granulocytes, but not in multinuclear giant cells and mononuclear inflammatory cells (Figure 1E). These IL-8-positive cells were exclusively found adjacent to the lens (Figure 1E). CD68-positive macrophages were diffusely present around the lens (Figure 1F). On the other hand, interferon  $\gamma$ -positive cells were scattered in the mononuclear inflammatory cells (data not shown).

## Discussion

In the present case, the intraocular inflammation appeared as ocular Behçet's disease and persisted for 4 years. The closure of pupil and the cataract formation prevented clinical observation during this period. Nonetheless, the histologic examination disclosed that the intraocular inflammation was caused not only by Behçet's disease but also by lens-induced uveitis. Diagnosis was based on the fact that multinuclear giant cells were present around the disrupted basement membrane of the lens and, further, that the fibrous membrane had formed on and beneath the lens, which are typical histological findings of lens induced-uveitis. It is possible that the persistent ocular inflammatory reaction had impaired the lens capsule, which eventually led to the subsequent lens-induced uveitis. The initial ocular findings and extraocular symptoms were specific to ocular Behçet's disease. The histologic findings of the enucleated eye and the diffuse

mononuclear and granulocytic infiltration in the atrophic retina and choroid were compatible with long-lasting Behçet's disease. It is possible that the persistent ocular inflammatory reaction had impaired the lens capsule, which led to the subsequent lens-induced uveitis.

IL-8 is a strong proinflammatory cytokine and is upregulated in various forms of ocular inflammation. The neutralizing antibody for IL-8 can inhibit the progression of intraocular inflammation in animals,<sup>3,6</sup> indicating that IL-8 can be a central player in human intraocular inflammation. In a *in vitro* study, virtually all cells can produce IL-8 in the presence of various stimuli; macrophages are supposed to be the major source of IL-8 *in vitro*.<sup>6,7</sup> In this study, nonetheless, IL-8 was exclusively found in the granulocytes, especially around the lens material, but not in the lymphocytes or macrophages, which were identified by the immunohistochemical study.

An immunohistochemical analysis is not the ideal method to quantify any protein and, therefore, the present results do not conclusively show that the macrophages do not produce any IL-8 in intraocular inflammation. The amount of IL-8 produced by macrophages might be under detectable levels using the present method. In addition, the amount of intraocular IL-8 might also change during different phases of inflammation.

Regardless of these various possibilities, the present results indicate that granulocytes might be a major source of IL-8 and that they, therefore, may play an important role in intraocular inflammation, including Behçet's disease complicated by lens-induced endophthalmitis. Further investigation is required to prove these hypotheses by a study of more cases.

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